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SENSITIVITY, SPECIFICITY AND ACCURACY OF THE ARABIDOPSIS ASSAY IN THE IDENTIFICATION OF CARCINOGENS

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16. ABSTRACT

The paper compares the sensitivity, specificity and accuracy of Arabidopsis multilocus assay with several other in vitro and in vivo bioassays. Sensitivity was defined as a percentage of the carcinogens correctly identified as mutagens. Specificity was calculated by multiplying with 100 the fraction of the number correctly identified "non-carcinogens"/total number of "non-carcinogens" unambiguously classified. Accuracy represents the fraction of the correct answers/ all unambiguous test, multiplied with 100. These parameters were estimated in Arabidopsis by testing 37 carcinogen/non-carcinogen pairs of chemicals. The results from this study were compared with those published by de Serres and Ashby 1981 (de Serres, F. J. and J. Ashby, Ed.s., 1981, Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program. Elsevier/North Holland, New York). The overall sensitivity of Arabidopsis assay was higher than any of the bioassays employed in the Internation Collaborative Program. This excellent performance of Arabidopsis may be due to very large number of loci scanned for the Arabidopsis assay and its intrinsic metabolic activation system.

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Sensitivity, Specificity and Accuracy of the Arabidopsis Assay in the Identification of Carcinogens.

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INTRODUCTION

Practically all human adults are conscious of the dangers of chemical hazards looming in the environment. Only a very small fraction of the population is cognizant, however, of the reliability of classification of the various agents as harmful or harmless to the human health.

Direct assessment of chemicals regarding carcinogenicity to human beings are impossible, and the theoretically best tests using animals are also impractical for large scale surveys. The direct assays with animals are too expensive, and because of their long duration they cannot keep up with the deluge of new compounds identified or synthesized continually. According to some estimates, about one new chemical becomes known every two minutes (10), and there are many among the over 6,000,000 compounds (11) accumulated in the recent past that have not been adequately examined regarding their biological properties.

Cancer has been afflicting mankind for centuries. Tumors were observed on 5,000 years old Egyptian mummies and on dinosaures which lived 18 million years ago. Fossil records indicate the presence of tumors on plants in the carboniferous strata dating back 50 million

years (20). The ancient Greek philosopher-physicians wrote extensively about human cancer, and they entertained the view that it is caused by environmental effects. Theodor Boveri in 1902 suggested "that malignant tumors might be the result of a certain abnormal condition of the chromosomes." Also, he found it "conceivable that in a species with a very small number of chromosomes, malignant tumors cannot appear at all." (Ref. 4, p. 46).

Auerbach and Robson (1944) have shown that the carcinogenic mustards are mutagenic to the low chromosome number Drosophila (3), and subsequently the mutagenic effects of carcinogens was demonstrated for a number of eukaryotes and prokaryotes (19). Nevertheless the relation between mutation and carcinogenicity did not receive universal recognition then (5) and some doubts still remain today (24; see also Mutation Research 99:73-9).

At present a wide variety of prokaryotic and eukaryotic short-term assays are available (9,21, see also Gene-Tox Reports in Mutation Research vols. 87 and 99). None of the short-term assays known today are capable of identifying correctly all the carcinogens (15). The problems with the proper identification of "noncarcinogens" is even greater because there are no valid criteria for defining what a noncarcinogen is. Very weak carcinogens are extremely difficult to distinguish from noncarcinogens because "spontaneous" incidence of cancer blurs the differences.

The majority of the short-term assays are designed to detect alterations in the genetic material (genotoxic effects) that are directly or indirectly correlated with the expression of neoplasia

or malignant growth. Most of these short-term assays miss carcinogenic agents which act through metabolic regulatory means (epigenetic effects).

The short-term assays differ also in their ability of activating procarcinogens to become proximate or ultimate carcinogens. Most of the prokaryotic assays have a rather limited activating power and rely on the activating enzymes provided through the microsomal fraction of rodent liver homogenates. Even several eukaryotic assays (e.g. yeast or animal cell cultures) benefit by the use of the exogenous microsomal enzymes. Although these supplements to the in vitro tests enhance the efficiency of identification of carcinogenicity, the exogenous material may not entirely duplicate the metabolic processes taking place in intact animals. Carcinogenicity depends on a variety of metabolic pathways (activations and detoxifications), and it is expected to involve structural and developmental processes which may be organ-specific (26). Therefore, theoretically the most "native" systems of detection are expected to be the most reliable. In practice, unfortunately, this expectation has not been realized.

An alternative to the biological tests would be the chemical definition of the molecular fragments which contribute to the assembly of carcinogenic compounds. Such an inquiry should be rewarding by computer assisted classification of chemicals and their active groups but at this time neither the statistical approach nor the considerations of the organic chemistry can provide reliable predictions (8).

THE PURPOSE OF THIS STUDY

If none of the short-term assays are capable of correctly identifying all the chemicals regarding carcinogenic properties, one would like to have a battery of tests which through complementary performance would identify as large a fraction as possible of all carcinogens. Similarly, one may develop a reasonably safe system for granting clearance to most of the noncarcinogens. The latter goal is actually more difficult to acheive because defining noncarcinogenicity is almost the same task as proving that there are no witches. The information provided by a battery of three hypothetical assays can be represented as shown in Table 1. Although none of the individual assays need to be perfect, the 2/3 majority of the correct answers may be acceptable for classification of the various carcinogenic agents. A larger battery, comprising several types of tests is expected to be more reliable than the one shown (Table 1); the larger number involves, however, more time and cost.

Within an international cooperative program (7) a maximum of 42 chemicals were tested by several modifications of the best established (15) bacterial mutation assays and a large number of other microbial and eukaryotic tests, including intact animals and in vitro studies in order to find the best complements to the Salmonella/S9 histidine reversion systems (7).

MATERIALS AND METHODS

The chemicals were selected to include representatives of some of the major classes of organic compounds. When possible a structurally closely related but presumably noncarcinogenic analog

was also chosen for each proven carcinogen. Among the compounds used were some widely but presumably harmless chemicals such as sucrose, ascorbic acid and methionine. Special consideration was given to the selection of batches of the compounds with the highest available purity.

The 25 carcinogens were classified as such on the basis of information obtained from reliable direct carcinogen assays with animals. This group included 4-nitroquinoline-N-oxide, benzidine, 4-dimethylaminobenzene (butter yellow), benzo(a) pyrene, β-propiolactone, 9,10-dimethylanthracene (carcinogenicity information had not been originally entirely convincing), chloroform, 2-acetylaminofluorene, dimethylcarbamoyl chloride, 2-naphythylamine, N-nitrosomorpholine, urethane, methylazoxymethanol acetate, DL-ethionine, hydrazine sulphate, hexamethylphosphoramide (HMPA), ethylenethiourea, diethylstilbestrol, safrole, cyclophosphamide, epichlorhydrine, 3-aminotriazole, 4,4'-methylenebis(2-chloroaniline) [MOCA], o-toluidine hydrochloride and auramine (technical grade).

The noncarcinogens belonged to groups I, II and III, indicating in this order the strength of evidence regarding their lack of carcinogenic activities. Group I contained only isopropyl-N-(3-chlorophenyl)carbamate. Group II included 4-dimethylaminoazobenzene-4-sulphonic acid Na salt, 1,1,1-trichloroethane, dimethylformamide, 1-naphythylamine, and diphenylnitrosamine. In Group III we find 3-methyl-4-nitroquinoline-N-oxide, 3,3',5,5'-tetramethylbenzidine, γ-butyrolactone, dinitrosopentamethylene tetramine, azoxybenzene and sucrose.

The third major group consisted of compounds which remained "unclassified" because of the lack of sufficient evidence regarding carcinogenic or noncarcinogenic properties: pyrene, anthracene, 4-acetylaminofluorene, L-methionine and ascorbic acid (sodium ascorbate).

The 61 assays included in the program are identified in Table 2. The description of the Arabidopsis embryo assays can be found on pages..... of this volume. The other assays are identified in more detail in reference 7. The Salmonella plate assays were basically similar or identical to the Ames test, albeit some of the laboratories used their own preferred protocols. One of the Salmonella assays (No. 14) used also the supplement norharman (abbreviated NH, SH-pyrido [3,4-b]indole), a compound which enhanced the mutagenicity of some chemicals and reduced that of others. The fluctuation assays were somewhat similar to the plate assays but used liquid media.

The prokaryotic repair assays utilized either Rec$^-$ bacteria or polA$^-$ Escherichia coli and scored the survival. Assay no. 26 was the so called "zoro" test. Lysogenic E. coli contained the λ prophage so constructed that the bacterial gal gene was fused to the cro repressor of the phage. The system included also the phage repressor cI. When the cI repressor is present the cro gene is shut off; when the cro gene is expressed cI is shut off. In the former case, on galactose medium in the presence of an indicator stain, white colonies are formed, in the latter case the colonies appear red. These two phenotypes of the colonies may result either because of a mutation in the cI gene (genotoxic effect) or by a proteolytic cleavage of the cI-coded repressor protein

(epigenetic effect). Assay no. 27 measured λ phage induction in E. coli.

The eukaryotic "in vitro" assays included both forward and reverse mutation, mitotic recombination, gene conversion, increase in aneuploidy, and genetic repair in yeast. Three assays used the criteria of unscheduled DNA synthesis (an indication of repair) in cultured human fibroblasts or HeLa cancer cells, respectively. Three assays measured the frequency of sister chromatid exchange in cultured Chinese hamster ovary cells. Chromosome aberrations were scored in a cultured rat liver epithelial-like cell (RL) system, and in Chinese hamster ovary cells in assays nos. 46 and 47, respectively. Forward mutation at the thymidine kinase locus was scored in mouse lymphoma cell line L5178Y (assay 48), in Chinese hamster ovary cells at the loci controlling resistance to azaadenine, thioguanine, ouabain, and fluorodeoxyuridine (assay no. 49), for thioguanine resistance in Chinese hamster ovary cell line CHO-K_1-BH_4 (assay no. 50), for hypoxanthine-guaninephosphoribosyl transferase deficiency in Chinese hamster cell line V79 (assay no. 5]), for diphteria toxin resistance (HF Dip^r) in human fibroblast culture (assay no. 52). Baby hamster kidney cell transformation was scored in BHK21 C13/HRC1 (assay no. 53) and in BHK-21 cells lines (assay no. 54), respectively.

A nongenetic criterion, displacement of polysomes from the endoplasmic reticulum of rat cells, was used by assay no. 29, and the nuclear enlargement of HeLa cell and human fibroblast was scored in assay no. 31.

The intact animal assays included three sex-linked recessive lethal tests with Drosophila using the FM6 stock (heterozygous for

multiple inversions and carrying the w, y and B markers), the Berlin K wild type carrying the y, mei-9^{a}, and mei-41^{D5} (thus deficient in excision and postreplication repair), and the standard Basc female system mated to Berlin K males, respectively (assays nos. 56, 57, 58). Assay no. 59 was involved in the determination of sister chromatid exchange in the bone marrow (and liver cells) of CBA/J male mice. Micronucleus tests were carried out on bone marrow cells of B6C3F$_1$ mice (assay no. 60), on bone marrow or erythrocytes of male ICR mice (assay no. 61), and on the bone marrow of CD-1 mice (assay no. 62), respectively. Mouse sperm abnormality was assayed on B6C3F$_1$/CRL animals.

The Arabidopsis assays were conducted in our laboratory in Columbia, Missouri, and the results have not been reported yet in detail, the outcome of the other assays are summarized on the basis of the publication of the final report (7) where the laboratories and investigators are identified.

RESULTS

Not all the laboratories involved in this project tested all the 42 chemicals and not all the tests permitted a definite conclusion regarding the activity of the individual compounds. In this summary, some assays were not included because of the incompleteness of the data. In the following, we will compare the performance of the single plant assay (Arabidopsis) with bacterial mutation, prokaryotic repair, a diverse group of in vitro eukaryotic assays and 8 intact animal assays (Table 2).

In Table 2 the results of each assay is shown by a filled circle if the classification agreed with the expectation based on direct carcinogenicity information. The open circles indicate that

the results were in disagreement with the expectation, the dots signal lack of data for a particular test and the question marks indicate that the information provided by the test was ambiguous The screened lines identify the compounds for which there was no evidence for carcinogenicity at the stage of the design of the program, and therefore we will consider these chemicals as "non-carcinogens". Thus if a particular assay classified them as inactive in inducing mutation or meeting other criteria of activity, the result is considered correct and is represented by a filled circle.

Ideally, Table 2 should have provided 42 x 61 = 2562 bits of useful information. Some of the chemicals were not tested by all assays and some of the results were ambiguous. Therefore in the evaluation only the reasonably certain pieces of information were considered. For the analysis of the data the classification of Cooper, Saracci and Cole (6) was adopted. Sensitivity of an assay was determined by multiplying with 100 the fraction of the number of carcinogens correctly identified/total number of carcinogens unambiguously tested. This index defined the power of identification of the true carcinogens (true positives). Specificity was calculated by multiplying with 100 the fraction of the number of correctly identified "noncarcinogens"/total number of "noncarcinogens" unambiguously classified. This index defined the power of identification of the "noncarcingoens" (true negatives). Accuracy represents the fraction of the correct answers/all unambiguous tests, mutiplied with 100. This estimate combines in a single figure the correct positive and correct negative data.

The sensitivity of a test defines thus the frequency of suc-

cessful identification of a particular group of carcinogens by an assay or by a group of assays. Obviously this parameter is not directly applicable to another group of chemicals. From the view point of health hazards this is still the most useful criterion of a good assay system.

The specificity index has more meaning from the view-point of the economist than from that of the biologist. It can be used to grant clearance to an industrially important compound but it will never prove that a chemical is absolutely innocuous to a mammalian system. Obviously, it is desirable to be aware of this index because modern societies are dependent on a large number of chemicals and some reasonable compromises are unavoidable.

The accuracy estimates may indicate the overall versatility and utility of an assay. Although it may be quite misleading too because two assays may have the same accuracy index yet one which is highly sensitive to carcinogens may classify as carcinogens a large number of "noncarcinogens", the other may acquire a high accuracy figure through failure of identifying many carcinogens but giving negative scores (i.e. "true" noncarcinogen rating) to most of the presumable "noncarcinogens".

Sensitivity

Table 3 summaries the sensitivity (true positive) indexes of the five major groups of assays to 25 carcinogens. The overall sensitivity of the 61 assays is disappointingly low; only 58% of the unambiguous answers were correct. Thus many dangerous compounds could have been misclassified on the basis of these results. Surprisingly,the intact animal tests involving mutational and cytogenetic criteria missed about two thirds of the compounds.

This can be considered particularly bad because most of these assays are relatively slow and expensive. The best assay systems of bacterial mutation performed about as well as the prokaryotic repair assays or the in vitro eukaryotic assays which are considered generally as inferior to the Salmonella/S9 and similar systems. Arabidopsis, the only higher plant for what data are shown, performed extremely well because 19/20 of the carcinogenic chemicals were properly identified.

Table 3 shows great variation in the facilities by which the different chemicals are identified. Some compounds, such as 4-nitroquinoline-N-oxide (1.), benzo(a)pyrene (7.), 2-acetylaminofluorene (15.), methylazoxymethanolacetate (26.) and epichlorhydrine (36) were detectable by an overall efficiency of about 80%. On the other hand chloroform (14.), ethionine (28.), diethylstilbestrol (33.) and 3-aminotriazole (37.) were all identified by only 25% or even less efficiency.

The data (Table 3) provide very little evidence that any type of assay would be particularly effective in identifying carcinogens in cases where the other types of tests are ineffective or show poor performance. Remarkably, the plant assay (Arabidopsis) seems to be an exception because it identified correctly 3 of the 4 carcinogens which were missed by the great majority of the other assays.

On the brighter side of the picture, it is gratifying to see that 5 of the carcinogens were identified correctly by 100% of the bacterial mutation assays.

Specificity

On the 17 "noncarcinogens" 63% of all the assays gave the

expected classification based on the original information (Table 4). The sensitivity of the intact animal assays was the highest (85), and Arabidopsis displayed the lowest grade (25). The three other types of assays were again close to each other and in between the two extremes represented by the intact animal and the Arabidopsis test, respectively. The noncarcinogens, 3,3'5,5'-tetramethylbenzidine (4.), γ-butyrolactone (10.), anthracene (12.), 1,1,1-trichloroethane (13.), dimethylformamide (18.), L-methionine (29.) and sucrose (41.) all scored better than 80. Five presumed "noncarcinogens", namely 3-methyl-4-nitroquinoline-N-oxide (2.), 4-acetylaminofluorene (16.), 1-naphthylamine (20.), diphenylnitrosamine (22.) and azoxybenzene (27.) obtained specificity rating below 50 and therefore they seem to be mutagenic (and possibly carcinogenic) in spite of the fact that the original information indicated otherwise. We may recall that no. 2 and no. 27 had the class III (the least certain) noncarcinogen classification. No. 20 and 22 were originally believed to belong to class II of the noncarcinogens, whereas no. 16 was considered as a compound for which the carcinogenicity information is inadequate. In addition, compound no. 6 scored only 45 by the bacterial mutation tests combined and it also appears to be a mutagen (and possibly a carcinogen).

The Arabidopsis embryo assay identified four of these six compounds as mutagenic and two of them were not tested. Obviously a reclassification, by taking into consideration the overall results of these 61 assays, would have improved substantially the specificity rating of the Arabidopsis assay and would have lowered the scores of the intact animal test systems.

Accuracy

The accuracy of the five major types of assays is provided in Table 5. In spite of its low specificity, the Arabidopsis assay displayed the highest accuracy. Among the other groups of assays, the accuracy of the bacterial mutation tests was the best. This confirms the generally recognized usefulness of these assays (15). The intact animal assays, as a group, appeared to be the least accurate.

If we rank the accuracy of individual types of assays, rather than groups of assays, a completely different order emerges (Table 6). The Arabidopsis test is surpassed in accuracy by four other assays, and surprisingly the bacterial mutation assays (the 12 Salmonella plate assays and the 3 fluctuation tests) occupy the 9th and the 10th positions with equal ratings. The human fibroblast assays using diphteria toxin resistance raises to the top. We must note, however, that this apparently superior performance may be due to the few compounds tested, and most of the carcinogens which are very hard or impossible to identify with the other assays, were not tested. Such a criticism does apply to the four other assays among the top five by accuracy ratings. The relatively low rating of the bacterial mutation assays is due to the fact that several of the carcinogens included in the project were selected on the basis of some known difficulties of identification with the best established assays (2). These 42 chemicals do not represent an entirely random sample of the compounds common in the human environment.

Complementarity of the Assays

For the purpose of selecting the best battery of assays, we must know which chemical group of carcinogens cannot be safely identified with the bacterial mutation assays that are the best established. Eleven carcinogenic compounds included in this project were generally or very frequently missed by the bacterial mutation assays as well as by the prokaryotic repair tests (Table 7). The overall sensitivities to these 11 compounds of the bacterial mutation assays, prokaryotic repair, eukaryotic systems and intact animal assays were 18, 37, 42 and 32, respectively. It is interesting to compare how the top six assays, so rated on the basis of overall accuracy, detected the activity of these compounds. The assay which appeared no. 1 in Table 6, correctly identified only a single out of the 9 which were unambiguously classified. Obviously such an assay cannot be employed successfully in a battery of tests involving prokaryotic assay systems. Also, the bacterial plate assay (19.) which was rated fourth, identified correctly only two of this group of chemicals. The Arabidopsis embryo test, the Saccharomyces reversion assay (32.), the Bacillus subtilis repair test (21.) and one of the two baby hamster kidney cell transformation assays (54.) showed 90, 78, 72 and 72, sensitivity, respectively. Also the Saccharomyces repair test (39.) which had rather low overall rank in Table 6, correctly identified 9 of these 11 carcinogens and thus displayed a sensitivity of 81 to these compounds. The performance of the sister chromatid exchange assay using Chinese hamster ovary cells (45.) was much better in sensitivity than the prokaryotic assays yet it identified correctly only 5 out of the 11 compounds. The Drosophila sex-linked recessive lethal assays

tested only 4 of the 11 compounds and missed 3 out of the 4, therefore they cannot be adequately classified but do not appear to show much promise regarding complementarity to the prokaryotic tests.

Metabolic Activation

All the prokaryotic assays and most of the in vitro assays, involving lower eukaryotes or cell cultures of higher animals, require the addition of activating systems for the proper identification of promutagens (procarcinogens). The intact animal assays rely on the metabolic system of the animal used. In the Arabidopsis assays, the chemicals were administered either in water or in dilute dimethylsulphoxide solution and no exogenous activating enzymes were used.

On the basis of the information provided by this study, it seems rather certain that the carcinogens 4-dimethylaminoazobenzene, benzidine, 2-acetylaminofluorene, benzo(a)pyrene, safrole, DL-ethionine, N-nitrosomorpholine, azoxybenzene, 4,4'-methylenebis (2-chloroaniline), 9,10-dimethylanthracene and auramine require activation for genetic effects. All of these compounds were found mutagenic, however, for Arabidopsis. Similarly the "noncarcinogens" 4-acetylaminofluorene and 1-naphthylamine generally required activation in prokaryotes to become mutagenic; in yeasts their effects was unclear but in Arabidopsis both compounds appeared mutagenic. It has been known for some time that higher plants can activate promutagens (12,13,25).

SUMMARY AND CONCLUSIONS

In 61 laboratories using a variety of prokaryotic and eukaryotic short-term assays a maximum of 42 chemicals were tested. The pur-

pose of the study was to identify the best battery of assays for screening carcinogens. None of the assay systems identified correctly all the proven carcinogens or "noncarcinogens". The individual assays were characterized by sensitivity (% of correct identification of carcinogens), specificity (% of correct identification of "noncarcinogens"), predictivity (% of carcinogens correctly identified among all the compounds tested) and hypersensitivity (% of compounds classified as carcinogens among all the compounds tested (Table 8).

From biological view-point, sensitivity is the most important criterion of any assay systems and this parameter is independent of the number of carcinogens included in the test. The sensitivity is, however, dependent on structure of the chemicals involved. Thus, the sensitivity estimates obtained for one group of chemicals cannot be safely extrapolated for another group even when exactly the same biological assay is used.

The specificity estimate is very important from industrial or agricultural view-points but frequently it is an indication of the insensitivity of an assay system in identifying mutagens (carcinogens) of low effectiveness. The data of Table 2 indicate that 3-methyl-4-nitroquinoline-N-oxide (2.), 4-dimethylaminoazobenzine-4-sulphonic acid Na salt (6.), 4-acetylaminofluorene (16.), 1-naphtylamine (20.), diphenylnitrosamine (22.) and azoxybenzene (27.) are mutagenic in the majority of tests. The specificity of the tests toward these six compounds was below 50 (Table 4). If these six compounds are reclassified as carcinogens, the sensitivity ratings of the assay systems may substantially change as shown in parenthesis in Table 8. Several other "noncarcinogens" such as

pyrene and ascorbic acid may also be suspect of being low efficiency mutagens.

The accuracy may be considered as a useful estimate of the overall efficiency of the various assay systems but this parameter must not be extrapolated for any test performance beyond this group of chemicals.

It was somewhat surprising that the highest overall sensitivity was displayed by a plant assay (Arabidopsis), yet the data were in agreement with a survey based on the information reported by several laboratories on 56 known or suspected carcinogens and neoplastic agents of which 49 (87.5%) were mutagenic (17). This good performance may be due to the very large number (thousands) of loci scanned for forward mutation (16).

It is somewhat disappointing that the overall sensitivities of the best established assays (15) failed to identify about 40% of the carcinogens. This low success may find explanation in the nature of the chemical sample; some of the compounds were selected on the basis of difficulties of identification by the prokaryotic assays (2).

One may look also at the data from a more optimistic viewpoint too because some of the best individual assays have performed far better than the group averages. The short-term assays have, however, some inherent problems. The metabolic systems (26) even among mammals is somewhat different and even larger differences exist between taxonomically distant organims. Purchase (14) noted that 2-naphthylamine is a potent carcinogen for man and three laboratory species of mammals but it failed to induce cancer in rats and rabbits. Also, among 250 compounds tested with mouse

and rat 8% were carcinogenic only to mice and 7% only to rats, whereas 44% were carcinogenic and 38% were noncarcinogenic to both (14). Tomatis et al. (23) indicated that according to epidemiological evidence hematite dust causes lung cancer in man but mouse, hamster, guinea pig and rat experiments all appeared negative. Similar observations were made also with arsenic. A recent and very extensive study in England found that 4-chloromethylbiphenyl, a well-proven potent bacterial mutagen, turned out to be a relatively weak mutagen in mammalian in vitro systems and inactive in intact animal assays including direct carcinogenicity assays (1).

Although during the last 20 years very substantial progress has been made in the rapid screening of carcinogens and mutagens, much basic research is needed on the mechanism of carcinogenesis and mutagenesis before we can expect further advance. Some approaches for the future have recently been outlined (22) but a much deeper understanding of the structural and functional organization of the genome is necessary (18) before a major breakthrough can be achieved.

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Table 1. A hypothetical battery of assays that is considered satisfactory albeit each member of the battery is unsatisfactory alone. Filled and open circles indicate correct and incorrect results, respectively.

		ASSAYS		
AGENTS		1	2	3
Carcinogens	A	●	●	○
	B	●	○	●
	C	○	●	●
Noncarcinogens	D	●	●	○
	E	●	○	●
	F	○	●	●

Table 2. Summary of the results of 61 assays with 42 chemicals. Filled and open circles stand for correct and incorrect results, respectively. Question marks indicate ambiguity and dots represent lack of data. The screen distinguished the data obtained with "noncarcinogens". The Arabidopsis data are original, the rest is based on reference no. 7.

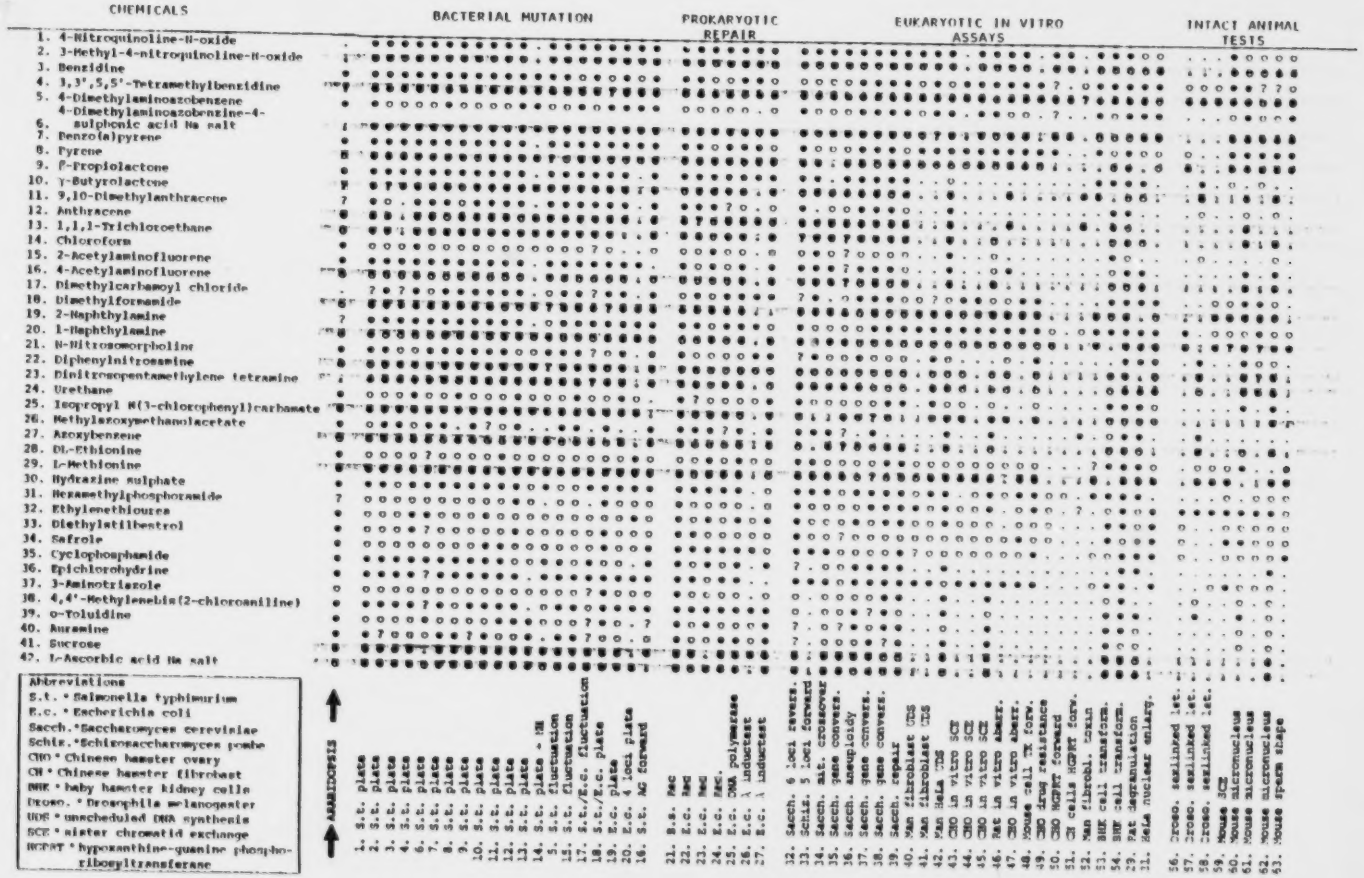

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TABLE 3. Sensitivities of the various types of assays to proven carcinogens. In the Arabidopsis assay +, - and ? stand for correct, incorrect and ambiguous results rewpectively.

	Chemicals	Arabidopsis Embryo Assay	Bacterial Mutation	Prokaryotic Repair	Eukaryotic Systems	Intact Animals	Total
1.	4-Nitroquinoline-N-oxide		100	100	91	20	89
3.	Benzidine	+	85	57	70	33	70
5.	4-Dimethylaminoazobenzene	+	45	0	65	40	47
7.	Benzo(a)pyrene	+	100	71	70	83	82
9.	β-Propiolactone	+	100	100	92	33	67
11.	9,10-Dimethylanthracene	?	88	60	70	0	71
14.	Chloroform	+	6	17	27	0	14
15.	2-Acetylaminofluorene	+	100	67	77	100	87
17.	Dimethylcarbamoyl chloride		81	71	63	40	68
19.	2-Naphthylamine	?	95	71	75	29	75
21.	N-Nitrosomorpholine	+	72	43	50	50	58
24.	Urethane	+	26	33	41	100	36
26.	Methylazoxymethanolacetate	+	77	100	90	100	86
28.	DL-Ethionine	+	11	29	33	40	25
30.	Hydrazine sulphate	+	80	83	73	0	71
31.	Hexamethylphosphoramide	?	11	17	48	88	38
32.	Ethylenethiourea	+	20	57	38	0	30
33.	Diethylstilbestrol	+	16	13	24	33	23
34.	Safrole	+	20	86	38	17	35
35.	Cyclophosphamide	+	84	57	78	100	78
36.	Epichlorohydrine	+	100	80	86	20	84
37.	3-Aminotriazole	-	5	17	46	0	18
38.	4,4'-Methylenebis(2-chloroaniline)	+	83	100	55	67	77
39.	o'Toluidine	+	29	43	60	0	39
40.	Auramine	+	46	57	50	0	47
	Total	95	59	58	60	37	58

Table 4. Specificities of the various types of assays to presumably noncarcinogenic chemicals. For symbols see Table 3.

	Chemicals	Arabidopsis Embryo Assay	Bacterial Mutation	Prokaryotic Repair	Eukaryotic Systems	Intact Animal Tests	Total
2.	3-Methyl-4-nitroquinoline-N-oxide		0	0	5	60	8
4.	3,3',5,5'-Tetramethylbenzidine	?	100	71	92	88	91
6.	4-Dimethylaminoazobenzine-4-sulfonic acid Na salt		45	71	71	100	64
8.	Pyrene	-	58	71	53	50	63
10.	γ-Butyrolactone	?	94	67	71	100	83
12.	Anthracene	+	89	67	92	100	87
13.	1,1,1-Trichloroethane	-	94	83	73	100	86
16.	4-Acetylaminofluorene	-	0	33	50	100	26
18.	Dimethylformamide	-	89	100	76	100	85
20.	1-Naphthylamine	-	30	50	36	100	42
22.	Diphenylnitrosamine	-	72	0	6	100	44
23.	Dinitrosopentamethylene tetramine		94	66	56	75	74
25.	Isopropyl N(3-chlorophenyl)carbamate	-	100	33	43	100	70
27.	Azoxybenzene	-	37	17	42	67	38
29.	L-Methionine	+	90	67	79	100	84
41.	Sucrose	+	100	100	75	100	92
42.	L-Ascorbic acid Na salt	-	84	67	58	100	74
	Total	25	69	56	57	85	63

Table 5. Accuracy of the five types of assays for the identification of carcinogenicity of chemicals. The body of the table shows the number of observations in accordance with expectation/total number of tests with conclusive results.

Chemicals	The groups of assays with their numbers: 1 Arabidopsis	20 Bacterial Mutation	7 Prokaryotic Repair	25 Eukaryotic Systems	8 Intact Animals	61 Total	Carcinogenicity	Success of Identification of Compounds
1. 4-Nitroquinoline-N-oxide	0/0	0/19	0/7	1/22	3/5	4/53	yes	8
2. 3-Methyl-4-nitroquinoline-N-oxide	0/0	19/19	7/7	21/22	2/5	49/53	no	93
3. Benzidine	1/1	17/20	4/7	16/23	2/6	40/57	yes	70
4. 3,3',5,5'-Tetramethylbenzidine	?	19/19	5/7	22/24	7/8	53/58	no	91
5. 4-Dimethylaminoazobenzene	1/1	9/20	0/6	13/20	2/5	25/52	yes	48
6. 4-Dimethylaminoazobenzene-4-sulphonic acid Na salt	0/0	9/20	5/7	15/21	4/4	33/52	no	64
7. Benzo(a)pyrene	1/1	19/19	5/7	16/23	5/6	46/56	yes	82
8. Pyrene	0/1	11/19	5/7	10/19	6/6	32/52	no	62
9. β-Propiolactone	1/1	18/18	7/7	11/12	1/3	38/41	yes	93
10. γ-Butyrolactone	?	17/18	4/6	10/14	3/3	34/41	no	83
11. 9,10-Dimethylanthracene	?	15/17	3/5	7/10	0/3	25/35	yes	71
12. Anthracene	1/1	16/18	4/6	12/13	1/1	34/39	no	87
13. 1,1,1-Trichloroethane	0/1	16/17	5/6	8/11	2/2	31/37	no	84
14. Chloroform	1/1	1/17	1/6	3/11	0/2	6/37	yes	16
15. 2-Acetylaminofluorene	1/1	18/18	4/6	10/13	2/2	35/40	yes	88
16. 4-Acetylaminofluorene	0/1	0/18	2/6	6/12	2/2	10/39	no	26
17. Dimethylcarbamoyl chloride	0/0	13/16	5/7	12/19	2/5	32/47	yes	68
18. Dimethylformamide	0/1	16/18	4/4	16/21	4/4	40/48	no	83
19. 2-Naphthylamine	?	18/19	5/7	18/24	2/7	43/57	yes	75
20. 1-Naphthylamine	0/1	6/20	3/6	8/22	5/5	22/54	no	41
21. N-Nitrosomorpholine	1/1	13/18	3/7	7/14	2/4	26/44	yes	59
22. Diphenylnitrosamine	0/1	13/18	0/6	1/17	3/3	17/45	no	38
23. Dinitrosopentamethylene tetramine	0/0	17/18	4/6	10/18	3/4	34/46	no	74
24. Urethane	1/1	5/19	2/6	7/17	2/2	17/45	yes	38
25. Isopropyl N(3-chlorophenyl)carbamate	0/1	19/19	2/6	6/14	1/1	28/41	no	68
26. Methylazoxymethanolacetate	1/1	10/13	5/5	9/10	1/1	26/30	yes	87
27. Azoxybenzene	0/1	7/19	1/6	5/12	2/3	15/41	no	37
28. DL-Ethionine	1/1	2/19	2/7	7/21	2/5	14/53	yes	26
29. Methionine	1/1	18/20	4/6	15/19	5/5	43/51	no	84
30. Hydrazine sulphate	1/1	16/20	5/6	16/22	0/4	38/53	yes	72
31. Hexamethylphosphoramide	?	2/18	1/6	10/21	7/8	20/53	yes	38
32. Ethylenethiourea	1/1	4/20	4/7	8/21	0/6	17/55	yes	31
33. Diethylstilbestrol	1/1	3/19	2/7	5/21	2/6	13/54	yes	24
34. Safrole	1/1	4/20	6/7	8/21	1/6	20/55	yes	36
35. Cyclophosphamide	1/1	16/19	4/7	7/9	1/1	29/37	yes	78
36. Epichlorohydrin	1/1	18/18	4/5	18/21	1/5	42/50	yes	84
37. 3-Aminotriazole	0/1	1/19	1/6	5/11	0/3	7/40	yes	18
38. 4,4'-Methylenebis(2-chloroaniline)	1/1	15/18	7/7	6/11	2/3	31/40	yes	78
39. o-Toluidine	1/1	5/17	3/7	6/10	0/2	15/37	yes	41
40. Auramine	1/1	7/15	4/7	5/10	0/2	17/35	yes	49
41. Sucrose	1/1	18/18	6/6	9/12	1/1	35/38	no	92
42. Ascorbic acid	0/1	16/19	4/6	7/12	1/1	28/39	no	72
Accuracy of Tests	68.8	63.0	57.1	58.9	55.0	60.3		

Table 6. Ranking the assays on the basis of accuracy judged by the results of this study involving 15 or more observations.

Rank	Assays	No. of Labs	Correct Answers / Number of Tests	Accuracy
1	Human cells, diphteria toxin resistance	1	12/16	75.0
2	Saccharomyces, reversion 6 loci	1	26/36	72.2
3	Bacillus subtilis repair	1	30/42	71.4
4	Escherichia coli plate assay	1	27/39	69.2
5	Arabidopsis, forward mutation, many loci	1	22/32	68.8
6	BHK cell transformation	2	52/79	65.8
7	Schizosaccharomyces, forward, 5 loci	1	21/32	65.6
8	CHO chromosome aberrations	1	13/20	65.0
9	Rat liver cell degranulation	1	22/34	64.7
10	HeLa unscheduled DNA synthesis	1	27/42	64.3
11	Salmonella/S9, histidine reversion	12	294/471	62.4
12	Bacterial fluctuation tests	3	73/117	62.4
13	Mouse, TK forward mutation	1	13/21	61.9
14	E. coli DNA-polymerase	1	25/42	59.5
15	Mouse micronucleus, intact animal	3	51/86	59.3
16	HeLa nuclear enlargement	1	14/24	58.3
17	E. coli - λ inductests	2	35/60	58.3
18	Saccharomyces aneuploidy	1	23/41	56.1
19	Saccharomyces gene conversion	3	63/113	55.8
20	Drosophila, sex-linked lethal	3	20/36	55.6
21	Saccharomyces repair	1	22/40	55.0
22	Mouse, SCE, intact animal	1	11/20	55.0
23	Rat, in vitro chromosomal aberrations	1	13/24	54.2
24	Mouse sperm shape	1	9/17	52.9
25	E. coli repair	3	63/120	52.5
26	Saccharomyces, mitotic crossingover	1	19/38	50.0
27	CHO, sister chromatid exchange	3	37/75	49.3
28	Human fibroblast UDS	2	20/42	47.6

Table 7. Experimental results with 11 carcinogens which are hard to identify by bacterial mutation assays as shown by these data. Figures in the body of the table represent sensitivity, and the signs +, -, o and ? indicate correct, incorrect identification or lack of information or ambiguity, respectively.

Carcinogens which are Hard to Identify by the Bacterial Mutation Assays	Groups of Assays				Top Six Assays Based on Overall Accuracy						Other Types of Assays Frequently Considered				
	Bacterial Mutation	Prokaryotic Repair	Eukaryotic Systems	Intact Animal Assays	Man, Fibroblast, Diphteria Toxin Resistance (52)	Saccharomyces Reversion at 6 Loci (32)	Bacillus subtilis Rec Reapir (21)	Escherichia coli, plate assay (19)	Arabidopsis Forward Mutation	Baby Hamster Kidney Cell Transformation (54)	Chinese Hamster Ovary Cell Chromosomal Aberrations (47)	Saccharomyces Repair (39)	Drosophila Sex-Linked Recessive Lethals (57)	Chinese Hamster Ovary Cell, Sister Chromatid Exchange (45)	Schizosaccharomyces, Forward Mutation (33)
5. 4-Dimethylaminoazobenzene	45	0	65	67	+	+	-	+	+	+	-	+	o	+	+
14. Chloroform	14	17	27	0	o	-	-	o	+	-	o	-	-	-	+
24. Urethane	26	33	41	100	o	+	+	+	+	+	o	+	o	-	-
28. DL-Ethionine	11	29	33	40	?	+	-	-	+	+	-	+	-	-	+
31. Hexamethylphosphoramide	11	17	48	88	o	+	+	-	?	+	-	+	+	+	-
32. Ethylenethiourea	20	57	38	0	o	+	+	-	+	+	-	+	o	-	-
33. Diethylstilbestrol	15	29	24	33	o	+	+	-	+	-	+	-	o	-	-
34. Safrole	20	67	38	17	-	+	+	-	+	+	-	+	o	-	+
37. 3-Aminotriazole	6	17	46	0	o	-	+	-	-	+	o	+	-	+	o
39. o-Toluidine	29	43	60	0	o	?	+	-	+	+	o	+	o	+	o
40. Auramine	58	57	50	0	o	?	+	-	+	-	o	+	o	+	o
Overall Sensitivity	18	37	42	36	.	78	72	.	90	72	.	81	.	45	50

Table 8. Synopsis of the parameters concerned with utility of the groups of assay systems. The numbers in parenthesis show sensitivies when some of the "noncarcinogens" were reclassified as carcinogens according to considerations described in the text.

Parameters	Arabidopsis Embryo Assay	Bacterial Mutation	Prokaryotic Repair	Eukaryotic Systems	Intact Animal Tests
Sensitivity	95	59	58	60	37
Specificity	25 (58)	69 (80)	56 (71)	57 (68)	85 (57)
Accuracy	69	63	57	59	55
Predictivity	67	73	67	67	82
Hypersensitivity	88	47	53	53	28